7.0 SUMMARY

7.1 INTRODUCTION

The non-ionizing and non-thermal radiofrequency electromagnetic field (RF-EMF) radiation, from cellphones, cell phone towers, and wireless communication, transmitters, falls under 800MHz to 300GHz.It has been a continuously growing ever since their introduction 30 decades ago and in the recent years, they have considerably increased in number. Most of the cell phone towers are found very close to where people live. They are especially mounted on rooftop of the school, residential, commercial buildings, colleges, hospitals and other working places. This type of radiation exposure around a cell phone tower, in residential areas, at distances of within 50 m to 300 m radius considered to be high radiation exposure zone. This radiofrequency electromagnetic field (RF-EMF) radiation is non-ionizing, non-thermal, in the frequency rangeof 900MHz to 1800MHz.

Cell phone usage and cell phone tower installation are a publichealth concern due to chronic exposure to low-level radiofrequency electromagnetic field (RF-EMF) radiation that pulse off the phone antenna. Cell phones and towers transmit electromagnetic waves in all directions, increasing the region of cells within the brain and bone marrow stem cells that are at risk for damage by RF/MW radiation penetrating the human body and animals, particularly blood cell and brain tissues. Although RF-EMF radiation can result in thermal damage if energy absorption rates are high, it is more likely that deleterious effects of RF-EMF radiation on cells of the bone marrow and brain would be due to non-thermal effects induced by lower intensities of exposure.In 2011, International Agency for Research on Cancer (IARC), part of World Health Organization (WHO), designated these exposures from cell phones as “possible human carcinogen”Class 2B. There are numerous short-term studies on the bio effects of RF-EMF radiation from cellphones on mesenchymal stem of human, guinea pig, mouse, rat and other mammals, but the long-term research of such exposures are inconclusive and studies on cell phone towers are scarce and almost non-existent in India. The electromagnetic radiations, known as electrosmog, cannot be seen, smelt or felt and no one would realise their potential harm over long periods of exposure until they manifest in the formof biological disorders. This invisible health hazard pollution (IHHP) is relatively a new environmental threat.

7.1.1 RF-EMF EXPOSURE STANDARD

The RF-EMF exposure will be lower from cell phone towers than from cell phones because the transmitter is placed directly against the head during cell phone use, whereas proximity to a

tower will be an ambient exposure at a distance **(Levitt, 2010);** nevertheless, it is continuous and involuntary. Exposure guidelines are set by International Commission on Non-Ionizing Radiation Protection (ICNIRP), National Radiological Protection Board (NRPB) and Institute of Electrica land Electronics Engineers (IEEE), adopted by many countries around the world **(Barnes & Greenebaum, 2007).** Antennae on cell tower transmit in the frequency range of 869-890 MHz forCDMA, 935-960 MHz for GSM900, 1805-1880 MHz for GSM1800, and 2110-2170 MHz for 3G.Wi-Fi frequency range is 2.4 GHz, WiMAX is 2.5-3.3 GHz and 4G LTE is 2.99 GHz. Government of India has adopted the ICNIRP standard of 450 mW/sq.m for 900 MHz and 920 mW/sq.m for1800 MHz, the most used frequencies for cell phones and cell phone towers in India. The power density (PD), defined as the energy flux per unit area in the plane of the field, decreasing with distance squared, is the variable that is used to measure the RF-EMF radiation from the cell phone towers, using well-calibrated, sensitive instruments **(Kumar, 2010 & 2013).**

7.1.2 MEASUREMENT OF ELECTROMAGNETIC RADIATION

The RF-EMF radiation measurements were carried out using hand-held radiation meters, which gave the precise power density measurements of the radiation source for a period of 6 months.

7.1.3 IN VIVO PURSUIT USING ANIMAL MODEL

The RF-EMF radiation exposure studies previously have been linked to cancer, changes in gene and DNA or protein expression, cell signaling, oxidative studies, apoptosis, blood brain-barrier and genotoxicity. There were numerous studies on cell phones and cell phone tower RF-EMF exposures that has demonstrated various biological effects, such as cell level damages, double- and single-strand DNA breaks, childhood leukemia, headaches, changes in sleep pattern, changes in cellular morphology and neural electrophysiology (Bioinitiative Report, 2014). The standards which govern the electromagnetic field level are considered safe by many countries around the world.

7.1.4 STEM CELL CULTURE AND EFFECTS OF RADIATION

The mesenchymal stem cells are very sensitive and specialized cell in animals and human. Stem cells, based on their potency, are classified as pluripotent stem cell, multipotent stem cell, unipotent stem cell and oligo potent stem cell. Sources of stem cells are mainly from animal and

human bone marrow, umbilical cord blood cell, blood, liver, kidney, skin, brain or nerve tissue and cardiac muscle. The adult bone marrow stem cell and umbilical cord blood are widely used, whereas embryonic stem cell land fetal stem cells are not used for cell culture. In this study, mesenchymal stem cells were isolated from the femur bone of guinea pig. The radiofrequency electromagnetic radiation-exposed guinea pig stem cells were isolated after exposure.

For medical research and experimental research as well as for research that explores the basic processes in the development of organisms and diseases, scientists often rely on animals. Implanting human cells into animals such as mice and guinea pig has long been common practice in order to test the safety and effectiveness of new drugs, procedures, experimental analysis such as some hazardous radiation and chemical exposure and medical devices before clinical testing in human. For stem cell research, scientists use animals to make sure the stem cells are able to incorporate into the tissue, do not cause any harmful consequences and function in concert with the rest of the body. Scientists can also trace the development and progression of certain diseases within an animal. By comparing experiments with guinea pig stem cells and normal stem cells, cancer or cell and DNA damaged stem cells were diagnosed.

7.2 REVIEW OF LITERATURE

Various studies worldwide have shown that the non-ionizing, non-thermal, low level RF-EMFradiations can cause leakage of blood brain barrier **(Persson BRR. *et al*., 1997),** damage tocell tissue and DNA **(Lai and Singh, 2005),** activate cellular stress response **(Velizarov S. *et al*., 1999; Leszczynski D. *et al.*, 2002),** generate reactive oxygen species (ROS) **(Lee S. *et al*., 2005;Nylund and Leszczynski, 2006),** interfere with cellular oxidative process altering antioxidant enzymes **(Kesari and Behari 2009),** suppress immune function by obstructing DNA repair **(Hallbergand Johansson, 2004),** up-regulate genes of apoptotic pathways **(Zhao TY. *et al*., 2007),** neuronal damage **(Salford LG. *et al*., 2003),** sperm head abnormalities **(Otitoloju AA. *et al*., 2010)** and reduced reproductive capacity **(Panagopoulos DJ. *et al*., 2010)**.

They are linked to brain tumours through epidemiology **(Hardell L. *et al*., 2007)** and poor mental health, depression, suicidal feelings and self-injury **(Schreier N. *et al.,* 2006; Oshima N. *et al*., 2012).** They are associated with breastcancer **(West J. *et al*., 2013)** and cancer (throat) in the vicinity of cell phone towers **(Kumar, 2010)** through case studies. Erratic migration and navigation difficulties of birds **(Warnke, 2007),** sparrow decline **(Balmori, 2005)** and colony collapse disorder in honey bees **(Kimmel S. *et al*., 2006;Kumar, 2011; Pattazhy, 2012)** in

relation to RF-EMF radiations have been investigated. According to World Health Organization (WHO) Research Agenda for Radiofrequency Fields (2010), RF-EMFresearch was urgently needed on high priority areas such as dosimetry, epidemiology and *invivo* animal studies. For instance, radiation from a cell phone penetrates deeper into the head of children **(Gandhi *et al*., 1996; Wiart *et al.,* 2008)** and certain tissues of a child's head, e.g., the bone marrow and the eye, absorb significantly more energy than those in an adult head **(Christ *et al*. 2010).**The Arnold Caplan, during the 1990s, defined MSCs as cells that could give rise to bone marrow and other tissues of human and animals, but also to cartilage, tendon and muscle **(Dennis et al., 1999; Caplan*et al*., 2006).**Stem cells obtained from guinea pig bone marrow (BMSCs) have been widely studied because of their relative easy access and differentiation to irradiated stem cell and normal stem cell of bone marrow **(Hung S.*et al*., 2002, Sekiya I, *et al*.,2002)**.MSCs can also acquire characteristics of non-mesodermal lineages, such as bone marrow cells, other tissue cells, *in vitro* and *in vivo* **(Pittenger *et al*., 1999; Schwartz *et al*., 2002; Verfaillie, 2002; Verfaillie *et al*., 2002).**Repeated exposure to radio frequency electromagnetic radiation (RF-EMF) caused changes in the brain of guinea pigs **(Gordon,1970; Baranski, 1972; Tolgskaya and Gordon, 1973).** All of the electronic equipments that we use in our daily life, without thinking how much we use or how often we use to create RF-EMF effects **(Ongel *et al*. 2009)**. Recent reports on follow in 6 months guinea pig treated with radiation confirm its adverse effects on brain growth and heart blood vessels damages **(Murphy BP. *et al*., 2001).**

7.3 AIM AND OBJECTIVES

The aim of this study is to decipher the impact of radiofrequency electromagnetic field (RF-EMF) radiation on bone marrow stem cell and histopathology of *Cavia porcellus* (guinea pig) through molecular cytology and histology.The objectives are to measure the RF-EMF radiation exposure on animals (guinea pig) and the guinea pigs were in the presence of RF-EMF radiation for 6 months. Then the animals were taken for experimental study, in which one group was away from radiation source, being the control, and the other being exposed at a distance of 20 meters. Observations were carried out in histopathological studies in various organs, stem cell culture and differentiation between irradiated stem cell and normal stem cell, DNA damage, and membrane damage in mesenchymal stem cell (MSCs).

7.4 MATERIALS AND METHODOLOGY

7.4.1 ANIMAL AND CAGE DESIGN TO RADIATION EXPOSING

The guinea pigs were maintained for a period of six months at two various direction and distances from the cell phone tower (RF-EMF sources) in Cage C1 at 5 m and Cage C2 at 20 m. The third cage was the sham-exposure Cage C, which contained the control group used to simulate the environmental conditions of RF-EMF-exposed animals, but in absence of RF-EMF exposure. At the end of the six months period, the animals were 185 days old (6.5 months). The difference in weight was significant at 5% level for the guinea pigs in Cage C2 at 20m exposure distance. The mean weight of female guinea pigs was more than male guinea pigs. Feeds were given libitum along with water. Bedding of sterilized husk was changed at intervals and the cage was maintained in a sterile manner.

7.4.2 STEM CELL CULTURE

The isolated mononuclear cells were counted and then seeded on to T25 and T75 cell culture flasks with the seeding density of 1×10^4 cells/cm^2. These cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with low glucose. The flasks were then incubated at 37˚C in 5% relative humidity and with a pressure of 60 psi for 48- 72 hrs to allow adherent cells to attach. The media and non-adherent cells were removed and washed with 10 ml PBS. The cell culture flask was then replaced with fresh cell cultured and continued to incubate. The medium was changed, once every three heterogenous cells appeared to diminish quickly through serial passage, particular, cells expressing haematopoietic and endothelial markers were lost in 2 passage.

7.4.3 FLOW CYTOMETRY

To confirm the immunophenotypic characteristics of MSCs, they were subjected to flow cytometry. 1×10^5 cells were harvested at the end of passage 2 after incubation with 0.25% trypsin – EDTA for 5 minutes. The cell suspension was washed with PBS before conjugation with the antibodies. 10µl of respective antibodies were added in the respective tubes containing 100µl of suspension. All the tubes were then incubated at room temperature for 20 minutes in dark. After incubation, cells were washed using BD FACS wash buffer to remove the unbound antibodies. After centrifugation, the supernatant was discarded and the pellet was further re-

suspended in 500µl of PBS and vortexed. The labeled cells were then analyzed by FACS cytometer using BD FACS Diva software (Becton Dickinson).

7.4.4 RT- PCR ANALYSIS

The Duncan–Hartley strain were maintained for a period of six months in specially designed cages, common environment conditions, exposed to RF-EMF radiation from a cell phone tower at two locations, one at a distance of 20 meters to opposite of the main beam of the antenna (C2) and another one at a distance of 5 meters beneath of antenna (C1). The non-exposed control animals (C) were kept and maintained in a location away from the RF-EMF exposure. Permission was sought from Institutional Animal Ethics Committee (IAEC) for animal research and appropriate care for the animals was undertaken as per guidelines of Committee for the Purpose and Control and Supervision of Experiments on Animals (CPCSEA). The molecular experiment was used by RT-PCR to identify the protein damages.

7.4.5 HISTOPATHOLOGY

The guinea pigs were subjected to histopathological study, and tissue of brain, heart, kidney, lung, liver, muscle, skin from both experimental C2 group and control C group of guinea pig were dissected taken out and immediately fixed in bouine's solution and embedded in paraffin. Tissue were sectioned at 5µ, processed and stained in Haematoxylin and Eosin for histopathological study.

7.5 RESULTS AND DISCUSSION

7.5.1 RF-RMF EXPOSURE SOURCE AND DESIGN OF ANIMAL CAGES

The six-month average day and night temperature (26$\pm$3.6℃) and relative humidity (69$\pm$13%) were noted at Cage C, Cage C1 and Cage C2. The six-month average RF-EMF radiation (Power Density in mW/sq.m) readings at Cage C were 0 mW/sq.m, Cage C1 was also recorded as0.408 mW/sq.m and Cage C2 was 0.842 mW/sq.m.

7.5.1.1 ANIMAL COMPARATIVE STUDY

The guinea pigs which were exposed for six months to radiofrequency electromagnetic field (RF-EMF) radiation when compared to the control guinea pigs were observed to have hair loss, lack of movement, sickness and changes in weight. In Cage C, the control animals were

observed to be healthy, with absences of hair loss and weight within normal limits. After six months, changes were found between the two groups of guinea pig organs.

7.5.2 STEM CELL CULTURE

Bone marrow-derived mesenchymal stem cells of two different groups of guinea pigs were shown to be morphologically distinguishable from non-adherent and adherent hematopoietic cells and other blood cells. The isolated mononuclear cells were cultured and were covered in plastic T25 flasks and T75 flasks, until the density of flask was $1\times10^4/1\times10^6$ cells/cm^2. The media was changed regularly, twice a week to remove the non-adherent cells. In the period of cell culture, within a week, adherent cells were obtained to heterogeneous cell population and the confluent stem cell was identified with focusing electron microscope. After two weeks, the homogeneous cell was observed and showed colony formation and fibroblast was shown, which later became 60-70% confluent cells and the confluent cells resembled mesenchymal stem cells.

7.5.3 FLOW CYTOMETRY ANALYSIS

The cells were investigated under *in vivo* conditions where tests were done to show the undifferentiated phenotype of MSCs by surface marker and gene expression profiles. The proliferation of MSCs were arrested without changes in morphology and differentiation capacity at second passage (P2) in two samples namely, control and exposed (C, C2). At confluence of 70 to 80%, the cultivated cells were detached and flow cytometry analysis was performed to verify the purity of cell population without any contamination. The purity was ensured, as there were no hematopoietic stem cells. Consequently, the following surface marker profiles were detected. Those markers were used for flow cytometry analysis CD90, CD105, CD29, CD34, and CD45. Later a cell aliquot was seeded out for further testing of their differentiation ability. Each investigated sample (C to C2) depicted homogenous cell population for the entire cell surface antigen (marker) and demonstrated that MSCs did not alter their physical and morphological properties during *ex vivo* expansion under the culture conditions. Flow cytometry and functional tests confirmed that more than 98.7% to 99.9% (Table 5.3) were destined to express the markers for hematopoietic lineages. Furthermore, the data did not show any statistical difference among the two samples, and overall percentage expression of these markers was quiet similar in the two samples.

The characterization of BMMSCs was ascertained based on the expression of specific markers. The cells were uniformly positive and highly expressive for the endoglin receptor CD 105+,

extracellular matrix protein CD90+ and β1-integrin CD29+. However, no detectable contamination of hematopoietic cells was observed. None of the cells expressed the markers for hematopoietic lineages. The leukocyte common antigen CD 45- from all the cells exhibited very low expression of epitopes, generally associated with hematopoietic progenitor cell, such as CD34- (4.75%), and (CD45- (7.40%). These results suggest that the cultures were virtually free of hematopoietic cells. Furthermore, all those samples showed very high expression (as per the mean) of epitopes, which were generally associated with MSCs, such as CD29 (99.65%), CD90 (99.60%), CD105- 99.85%, CD34 (-) – 4.75, CD45- (7.4%).

7.5.4 RT-PCR ANALYSIS

Real Time Polymerase Chain Reaction (RT-PCR) was done to profile the gene expression and fold change value differences of six genes of interest, following genes GFAP, FOS, TNF- α, HSB1, VEGF and TGF-beta with housekeeping gene (GAPDH) from the bone marrow stem cell (BMSCs) samples of RF-EMF exposed at Cage C2 and sham-exposed guinea pigs in Cage (C). The data was analysed using software Realplex 2.2 (Eppendorf). Transcript levels were normalized to glyceraldehyde-3 phosphate dehydrogenase (GAPDH) levels. To evaluate the gene expression in particular genes and the genes list were given above, after PCR melting curves were acquired and confirm the specificity of the amplified products. In RT-PCR, a standard curve based on cycle threshold (Ct) values were applied to evaluate gene expression, and the cycle threshold (Ct) values were acquired above 30 cycles and one did not amplify, so all genes showed positive results.

7.5.5 HISTOPATHOLOGY OF GUINEA PIGS

The radiofrequency electromagnetic field (RF-EMF) radiation-exposed guinea pig (C2) brain tissue section showed gliosis and observed effects at a molecular level in brain tissues. Irradiated guinea pig kidney tissues were observed with hypercellular glomeruli changes, central vein dilatation, widening of sinusoids and spilling of lymphocytes in the hepatocytes from the portal triad. In control group, heart tissues did not show damages, but revealed thinning of cardiomyocytes. As shown in microscopic examination, only small amount of cardiomyocytes with fibrin thrombi were observed. The lungs were observed to have thick walled blood vessels, areas of consolidation and inflammatory infiltrate of the lung parenchyma. A large oval-to-round cells with eccentric polar nuclei with marked peripheral accumulations of chromatin and in the skin with hyperkeratosis, increased pilosebaceous units and oedematous collagen in the upper dermis.

FUTURE SCOPE

Due to the overcrowding wireless signals from communications technologies, the air is being altered in unprecedented ways that have enormous consequences for life on earth. The future of communication technology is pervasive and ubiquitous computing, completely dependent on the non-ionising RF-EMF radiation, where people will live in an electronic environment enabled by wireless devices, which will help them carryout their everyday life activities. Devices will grow smaller, getting integrated into the environment and the technology will become invisible. Research tailor-made to Indian lifestyle, diet and environmental conditions, high-throughput screening techniques and mechanism of action, epidemiology data on cancer cases around cell phone towers, dosimetry measurements with sophisticated sensitive instruments and public awareness about the responsible use of cell phone technology need to be carried out in the coming years, as the environment gets saturated with RF-EMF radiation, coupled with rising temperature. The electromagnetic radiation effects on stem cell and bone marrow so bone cancer and stem cell cancer identified in molecular analysis. The government and industry should actively involve in addressing concerns about the installation of cell phone towers in densely populated areas with substandard infrastructure. One must exercise caution in dealing mobile telephony, in order to enjoy its immense benefits, while keeping a cautious eye as to where one lives, away from the presence of a cell phone tower (>20 m) and how one uses the wireless devices, especially the women, children and the elderly. This radiation of mobile telephony has harmful effects on different types of stem cells, especially embryonic stem cell, bone marrow stem cells.